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Effect of BAP (6-Benzyl Amino Purine) Concentration on Growth Microcutting of *Nepenthes ampullaria*

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Abstract

Conventionally, cultivations of Nepenthes are conducted by using seeds, cutting, and filial separation. However, there are many obstacles come both from time and technical aspect. In vitro culture is an alternative way for cultivating *Nepenthes ampullaria* (Jack,). One of a technique of in vitro culture is micro cutting. BAP (6-benzyl amino purine) growth regulator could be added to optimize the growth of *N. ampullaria* microcutting. The purpose of this research was to determine the effect of BAP on the growth of Nepenthes microcutting. This research was done experimentally using Completely Randomized Design (CRD). BAP treatment consisted of 5 concentrations: 0; 0.5; 1; 1.5; 2 (ppm), each treatment were multiplied 4 times. The parameters observed were: a time of bud initiation, time of root initiation, total of leaves, total of new buds, total of roots, length of leave, length of root, and height of bud. The data obtained were analyzed with Analisis of Variance and continued with 5% and 1% LSD (Least Significant Different) test. The result showed that addition of BAP affected the growth of *N. ampullaria* microcutting in total leaves, length of leave, and total of buds. LSD test proved that 0.57 ppm of BAP was optimal concentration to increase total buds, whit the value reached of 3.86. Here, we found that BAP can be utilized to enhanche *N. ampullaria* growth on in vitro culture. The benefit of this study is to conserve *N. ampullaria* in vitro using BAP at 0.57 ppm.

How to Cite

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INTRODUCTION

Nepenthes are rare and unique ornamental plants that have pitcher on the ends of the leaves (Sari *et al.*, 2015; Handayani & Astuti, 2005; Schulze *et al.*, 1997). The largest number of *Nepenthes* spp. species in Indonesia is on the island of Sumatra (Lestari *et al.*, 2018) In Indonesia, Nepenthes is a protected plants under the Law No. 5 of 1990 (Sukmadiyaja *et al.*, 2009). However, these plants are rare and difficulty to be found in the nature. Therefore, conservation efforts need to be done to overcome the extinction of Nepenthes. This conservation effort can be made through cultivation and plant breeding (Sari *et al.*, 2015).

According to Sukamto *et al.* (2011) *N. ampullaria* multiplication methods are often used are by seeds, cuttings, and separation of tillers. Wahyuni *et al.* (2017) has noted that plant tissue culture methods or in vitro culture can be used as an alternative solutions to increase productivity, such as micro-propagation. In vitro culture is the process of multiplying cells, tissues, organs, or protoplasts with sterile techniques (Nasir, 2002). One of the in vitro culture techniques is micro cuttings. Nepenthes propagation through micro cutting techniques can be done by culturing stems or shoots (Devi *et al.*, 2013). One of the factors that influence the success of in vitro culture is growth regulating substances (Lestari, 2011). Sayekti (2007) states that the growth regulating agent in the form of BAP (6-benzylaminopurine) from the cytokinin group can stimulate the formation of more buds. Giving BAP 0-1 ppm BAP could increase the number of leaves and buds on micro-culture of *N. mirabilis* (Dinarti *et al.*, 2010)

So far, no scientific repots were available on the effect of BAP addition on the growth of *N. ampullaria* in vito micro-cutting culture. Here we made experiments about the effect of different concentration of BAP on *N. ampullaria* growth. The aim of this research was to know the effect of BAP on the growth of micro cuttings and to determine the best concentration of BAP for the growth of *N. ampullaria* in vitro micro cuttings. The results of this research are expected to provide scientific information about the best technique of *N. ampullaria* propagation, as well as knowing the best concentration of BAP on the growth of *N. ampullaria* in vitro micro cuttings. The benefit of this research is providing new technique on *N. ampullaria* culture, especially the utilization of BAP on micro propagation of *N. ampullaria*.

METHODS

This research was done in Plant Physiology Laboratory, Biology Faculty of Jenderal Soedirman University for about four months starting from January, 17 until April, 29 2018. An experimental method was used during the research with Completely Randomized Design (CRD). The treatments consisteds of five different BAP concentrations that are 0.0; 0.5; 1.0; 1.5; and 2.0 ppm, respectively. Each treatment was multiplied four times.

The ½ MS was used as planting media. In the initial step, it was made a stock solution of planting media. The stock solution was made by weighing of the chemicals according to the composition of ½ MS media. The planting media composed of 50 ml/1 macronutrient stock solution, 0.5 ml/1 micronutrient stock, 0.5 ml/1 iodine stock, 0.25 ml/1 vitamins, and 1 ml/1 Fe-EDTA stock. The solution was mixed and homogenized with a stirrer, then added with distilled water up to a total volume of 500 ml. Ready stock of planting media solution was divided into five erlenmeyer tubes. The growing media in each tube was added with five different concentrations of BAP, they were 0.0; 0.5; 1.0; 1.5; and 2.0 ppm, respectively. Further, 200 ml distilled water and 4 grams of sugar were added to each treatment media. The pH of the media were adjusted between 5.83 and 5.85. The solution was homogenized with a stirrer then added with 1.6 grams of agar so that each treatment media and boiled. Each treatment media was poured on four culture bottles, then sterilized in Autoclave at 121 ºC and 2 atm pressure.

Planting the *N. ampullaria* explants was done by choosing the uniform explants. The explants were cut into four segments by removing apical shoots. The explants were grown in the treatment media, each culture bottle consisted of one explant. Culture bottles containing explants were stored on a culture rack with a temperature of 20 - 24 °C.

The observed variables were shoot initiation time, number of shoots, number of leaves, longest leaf length, root initiation time, number of roots, length of root. The obtained data were analyzed using Analysis of Variance (ANOVA) or F test, with 95% and 99% confidence levels followed with the LSD test.

RESULT AND DISCUSSION

The Effect of BAP on bud initiation on *N. ampullaria*

The results of ANOVA showed that different concentrations of BAP did not significantly affect the bud initiation. It was suspected that the number of endogenous cytokines in meristem was sufficient for bud growth, so the addition of exogenous cytokines in the form of BAP had no significant effect. Based on the results of the average bud initiation times, it appeared that the more concentrated BAP given, then initiation of buds tended to be slower. This statement is supported by Alitalia (2008), that the bud initiation process requires low BAP concentration. The giving of BAP concentrations that are too high will damage the tissue so that the process of shoots formation and cell enlargement will be inhibited.

The effect of BAP on *N. ampullaria* number of Buddings

ANOVA showed that supplementation of BAP on ½ MS culture media had a significant effect on buds' number after 4 weeks, 8 weeks, and 12 weeks observation. Since BAP is known to have stimulating effect on cell division and morphogenesis thus promote the development of new buds. According to Hutchison & Kieber (2002), cytokinins encourage cell division by accelerating G2-mytosis transition. Given that cytokinins can increase protein synthesis rate. Increasing in protein synthesis promote the development of new tissue which in turn followed by the development of new buds.

LSD test result shows that the addition of BAP to a certain concentration promote the development of new buds but after the optimum concentration it will show the opposite effect instead (Table 1). Regression test on BAP level and a number of buddings showed quadratic function with the optimum buddings at 1.2 ppm of BAP at 4 weeks having maximum budding value of 1.54, at 0.78 ppm of BAP at 8 weeks having maximum budding value of 2.93, and 0.54 ppm of BAP at 12 weeks having maximum budding value of 3.86 (Figure 1). To achieve a similar effect on a budding, the amount of BAP tended to be lower on older explant. The metabolism of an older plant produced more endogenous cytokinin thus minimizing the amount of exogenous cytokinin needed to stimulate budding. The addition of BAP could increase protein synthesis thus promote cell division and cell differentiation for budding formation. According to Sayekti (2007), BAP provides a statistically significant different result on N. alata budding. BAP gave the most effective effect at 0.5 ppm.

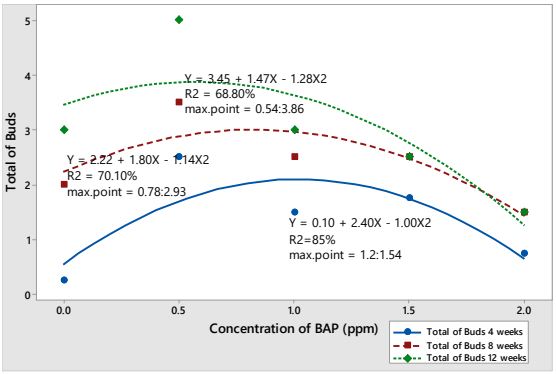

Figure 1. Regression curve of BAP - N.ampullaria budding relation

The effect of BAP on *N. ampullaria* leaflets development

Based on the result of ANOVA, BAP provided a statistically significant different effect on *N. ampullaria* leaflet development at 4 weeks, 8 weeks, and 12 weeks observation. Since BAP promote cell division, it promotes leaflet development as well. This claim was supported by Dinarti *et al.* (2010) that BAP on MS media provide a statistically significant different effect on the number of *N. mirabilis* leaflet, BAP concentration within 0 - 1 ppm could increase the number of

Table 1. LSD Test The Effect of BAP on Buddings at 4, 8, and 12 weeks

Treatmen	Number of Bud after 4 weeks	Number of Bud after 8 weeks	Number of Bud after 12 weeks
BAP 0.0 ppm	0.25 ± 0.50 a	2.00 ± 0.81 ab	3.00 ± 1.41 ab
BAP 0.5 ppm	2.50 ± 0.57 b	3.50 ± 1.00 b	5.00 ± 1.41 b
BAP 1.0 ppm	1.50 ± 1.29 ab	2.50 ± 0.57 ab	3.00 ± 1.15 ab
BAP 1.5 ppm	1.75 ± 0.57 ab	2.50 ± 0.57 ab	2.50 ± 0.57 a
BAP 2.0 ppm	0.75 ± 0.95 ab	1.50 ± 0.57 a	1.50 ± 0.57 a
LSD test 0.05	0.86	0.53	1.28

Note: value followed by the same letter in one column showed non significant different effect at α 0.05

leaflet.

LSD test showed that BAP promoted leaflets development to be better than in control group. Leaflet development rate increases as the giving of higher concentration BAP, but at some point, it goes down (Table 2). Regression test is resulting in quadratic function with the optimum BAP level at 0.68 ppm of BAP concentration and maximum leaflet of 11.43 at 4 weeks, at 0.64 ppm of BAP concentration and maximum leaflet of 14.76 at 8 weeks and at 0.68 ppm of BAP concentration and maximum leaflet 11.43 at 12 weeks (Figure 2). This condition is suspected because BAP can increase cell division, so that increasing the concentration of BAP given allows more cell division, thus increases leaflet development as well. Leaflet development process requires low BAP; BAP that exceeds the optimum level can decrease the number of a leaflet. Dinarti *et al.* (2010) stated that leaf development on in vitro culture was affected by cytokinin. Low concentration of BAP on culture media promoted more leaflet development while a high concentration of BAP, adversely, slowing down the growth and interrupting explant physiology.

Devi *et al.* (2013) stated that organogenesis (organ development) required cytokinin (BAP) in low concentration. Low BAP concentration enhanced protein synthesis thus accelerate cell division and organ development. Nursetiadi *et al.* (2016) added that BAP in culture media stimulates meristem cells in explant to multiply thus affecting the development of bud and leaf.

Based on the correlation test, it was found that there was a correlation between *N. ampullaria* growth with the observed parameters. This is indicated by the correlation of the number of shoots with the number of leaves with r2 = 0.52, which means that a number of leaves is related to the number of buds formed, the more number of buds formed, the more number of leaves will form. This can be seen in the BAP 0.5 ppm treatment, that the increase in the number of buds can encourage the addition of the number of leaves, the highest number of leaves at 12 weeks reach 17 leaves (Figure 3). These results are consistent with Intias (2012) statement that the development of leaves begins with the development of buds, the buds will elongate and form leaves. A high number of shots will produce a high number of leaves. Besides, the use of higher BAP can stimulate the growth of young leaves to be used as photosynthetic sites.

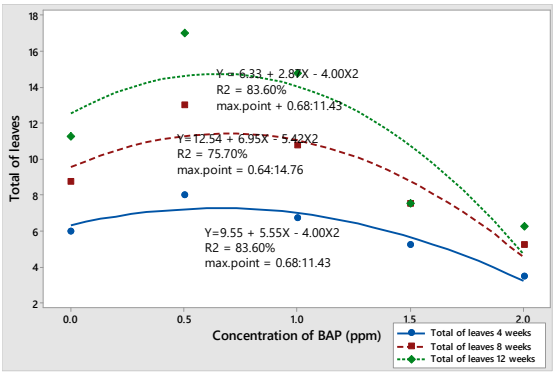

Figure 2. Regression curve of BAP - N.ampullaria budding relation

Figure 3. *N. ampullaria* after treatment with BAP 0,5 ppm (A) 4 weeks; (B) 8 weeks; and (C) 12 weeks.

The effect of BAP on *N. ampullaria* longest leaf length

The results of ANOVA showed that the addition of BAP had a very significant effect on the length of longest leaf on the observation after 12 weeks, because the addition of BAP plays a role in cell division and cell elongation so that the leaf

Table 2. LSD test the effect of BAP on leaflet development at 4, 8 and 12 weeks

Treatment	Number of leaf after 4 weeks	Number of leaf after 8 weeks	Number of leaf after 12 weeks
BAP 0.0 ppm	6.00 ± 1.41 ab	8.75 ± 3.50 ab	11.25 ± 2.06 abc
BAP 0.5 ppm	8.00 ± 0.81 b	13.00 ± 1.15 b	17.00 ± 3.74 c
BAP 1.0 ppm	6.75 ± 1.25 ab	10.75 ± 3.30 ab	14.75 ± 4.99 bc
BAP 1.5 ppm	5.25 ± 0.50 ab	7.50 ± 1.29 ab	7.50 ± 1.29 ab
BAP 2.0 ppm	3.50 ± 3.30 a	5.25 ± 3.86 a	6.25 ± 3.77 a
LSD test 0.05	3.31	2.19	12.60

Note: value followed by the same letter in one column showed non significant different effect at α 0.05

length will increase. This is in line with the statement of Lan *et al*. (2009) that BAP is a synthetic cytokinin which is important in regulating cell division, besides it can also stimulate leaf growth so that the number of leaves and length of the leaves increases.

Table 3. LSD test the effect of BAP on a longest leaf at 12 weeks

Tretment	longest leaf (mm)
BAP 0.0 ppm	12.75 ± 4.40 ab
BAP 0.5 ppm	19.50 ± 5.50 bc
BAP 1.0 ppm	22.25 ± 2.75 c
BAP 1.5 ppm	14.75 ± 4.43 ab
BAP 2.0 ppm	11.25 ± 1.25 a
LSD test 0.05	11.2

Note: value followed by the same letter showed non significant different effect at α 0.05

LSD test results show that the higher the BAP concentration, the length of the leaf will increase when compared with the control, then at a certain concentration it will decrease (Table 3). Regression test showed that BAP in the media gave a quadratic regression, the optimal concentration of BAP to increase the number of leaves was 0.83 ppm with a maximum leaf length of 20.77 mm (Figure 4). These results are following Alitalia's (2008) study result that the addition of BAP 1 ppm can increase leaf length to 19.8 mm, but BAP beyond the optimal limit can inhibit leaf elongation.

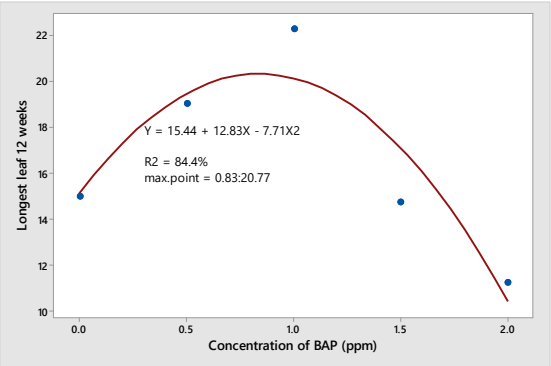

Figure 4. Regression curve of the relation of BAP and longest leaf of *N. ampullaria*

The need for cytokinins for leaf elongation is higher when compared to cytokinin needed for leaf initiation. This is because in leaf elongation there is a process of accelerating cell division to the size of the leaves during the leaf growth stage. The process of cell division for leaf elongation requires cytokinins and high energy, this energy can be obtained from carbohydrates in the media. This statement is supported by Sari *et al*. (2015) that BAP in high concentration was effective for leaf elongation.

The effect of BAP on plantlet height

The results of ANOVA showed that the addition of BAP did not significantly affect the plantlet height, because *N. ampullaria* explants are thought to have endogenous cytokinins which are sufficient for explant growth so that the need for cytokinins for explant growth is sufficient. Besides, the height of plantlets is also influenced by the genetic factors of the explants themselves, where *N. ampullaria* has a shorter height compared to the height of other Nepenthes. This is in line with the statment by Sari *et al*. (2015), that the growth of *N. ampullaria* is quite slow and genetically *N ampullaria* relatively shorter compared to other types of Nepenthes.

Based on the results of the average height of *N. ampullaria*, the increasing concentration of BAP given tendeds to increase explant height compared with the control group, then decreases at a particular concentration. The addition of BAP to 1 ppm tend to increase explant height, but BAP> 1 ppm can reduce the explant height. This is consistent with Alitalia's (2008) statement that in order to obtain maximum height in *N. miriabillis* explants, a relatively low concentration of BAP was required from 0 - 1 ppm BAP.

The effect of BAP on root initiation, number of root and the longth root

BAP on ½ MS media did not resulted in roots development in *N. ampullaria*. Since BAP was meant to stimulate shoot morphogenesis, while root development requires the certain ratio of cytokinins and auxin. When cytokinin is higher then shoot will be formed, conversely, roots will formef when auxin level is higher than cytokinin (Zulkarnain, 2009). In addition to it's genetic aspect which cause relatively slow root development in *N.* ampullaria. This condition is in line with statement of Yudhanto & Wiendi (2015), that BAP did not stimulate root development on *N. mirabilis*. According to Pratama *et al*. (2014), cytokinin added in media tend to enhance shoot and leaf development but decrease root development and explant height. Sayekti (2007) stated that naturally, Nephentess grows with not so many roots which indicated their low dependecy on nutrient absorption through roots.

N. ampullaria is a rare plant, but it can be developed with micro shoot cuttings. In this rese-

arch, micro shoots can be best grown in invitro with ½ MS media and the addition of BAP 0.57 ppm. The benefits and contribution of research for the science are to be used for the development of *N ampullaria* so that the plants will be abundant. This result can also be applied in farm to propagate *N.ampullaria*, so people do not need to collect *N.ampullaria* from the nature since it can be provided in high number by in vitro culture with BAP addition.

CONCLUSION

Based on the result and discussion above, it can be concluded that BAP plant regulatory hormones could enhance the growth of *N. ampullaria* explant, specifically on the number of leaf, length of leaf and the number of buds. BAP 0.57 ppm was the optimum concentration to increase the number of buds which was resulted in 3.86 buds.

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